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ABCB6 Mutations Cause Ocular Coloboma

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Ocular coloboma is a developmental defect of the eye and is due to abnormal or incomplete closure of the optic fissure. This disorder displays genetic and clinical heterogeneity. Using a positional cloning approach, we identified a mutation in the ATP-binding cassette (ABC) transporter *ABCB6* in a Chinese family affected by autosomal-dominant coloboma. The Leu811Val mutation was identified in seven affected members of the family and was absent in six unaffected members from three generations. A LOD score of 3.2 at $\theta = 0$ was calculated for the mutation identified in this family. Sequence analysis was performed on the *ABCB6* exons from 116 sporadic cases of microphthalmia with coloboma (MAC), isolated coloboma, and aniridia, and an additional mutation (A57T) was identified in three patients with MAC. These two mutations were not present in the ethnically matched control populations. Immunostaining of transiently transfected, Myc-tagged *ABCB6* in retinal pigment epithelial (RPE) cells showed that it localized to the endoplasmic reticulum and Golgi apparatus of RPE cells. RT-PCR of *ABCB6* mRNA in human cell lines and tissue indicated that ABCB6 is expressed in the retinae and RPE cells. Using zebrafish, we show that *abcb6* is expressed in the eye and CNS. Morpholino knockdown of *abcb6* in zebrafish produces a phenotype characteristic of coloboma and replicates the clinical phenotype observed in our index cases. The knockdown phenotype can be corrected with coinjection of the wild-type, but not mutant, *ABCB6* mRNA, suggesting that the phenotypes observed in zebrafish are due to insufficient *abcb6* function. Our results demonstrate that *ABCB6* mutations cause ocular coloboma.

Introduction

Ocular coloboma (MIM 120200) is a developmental defect of the eye and results from an abnormal or incomplete fusion of the optic fissure. The defect can affect areas of one or both of a patient's eyes, including the cornea, iris, ciliary body, lens, choroid, retina, and optic nerves.[1] Clinically, coloboma is often associated with microphthalmia and anophthalmia in the eye or occurs as part of complex malformation syndromes.[1,2] The prevalence of coloboma ranges from 0.5–7.5 per 10,000 births.[3,4] Approximately 10% of childhood blindness is caused by severe colobomatous malformations.[5] Coloboma can be caused by a genetic mutation or by toxic environmental factors.[1] At least 27 genes have been implicated in syndromes involving coloboma,[1,6–12] and *PAX6* (MIM 607108), *SHH* (MIM 600725), *GDF3* (MIM 606522), and *RBP* (MIM 180250) have been shown by linkage and mutational screening to cause isolated coloboma.[7,13,14] Among the 27 coloboma-associated genes, *CHX10* (MIM 142993), *MAF* (MIM 177075), *SOX2* (MIM 184429), *OTX2* (MIM 600037), and *RAX* (MIM 601881) can undergo mutations that result in ocular disorders including microphthalmia, cataracts, and microcornea. Mutations in other genes of this group might lead to other birth defects in addition to ocular coloboma.[1,6–12,15] Current studies demonstrate that all genes associated with coloboma play important roles in the early development of the human body, especially in the development of the CNS, including that of the eye. To date, mutations in the genes described above can explain approximately 50% of coloboma cases. Therefore, additional genetic mutations responsible for a coloboma phenotype still need to be identified.

Subjects and Methods

Ethics Statement

All procedures used in this study conformed to the tenets of the Declaration of Helsinki. All protocols were approved by the institutional review boards and ethics committees of Beijing University, Sichuan Academy of Medical Sciences Affiliated Hospital and Sichuan Provincial People's Hospital, and the Aravind Medical

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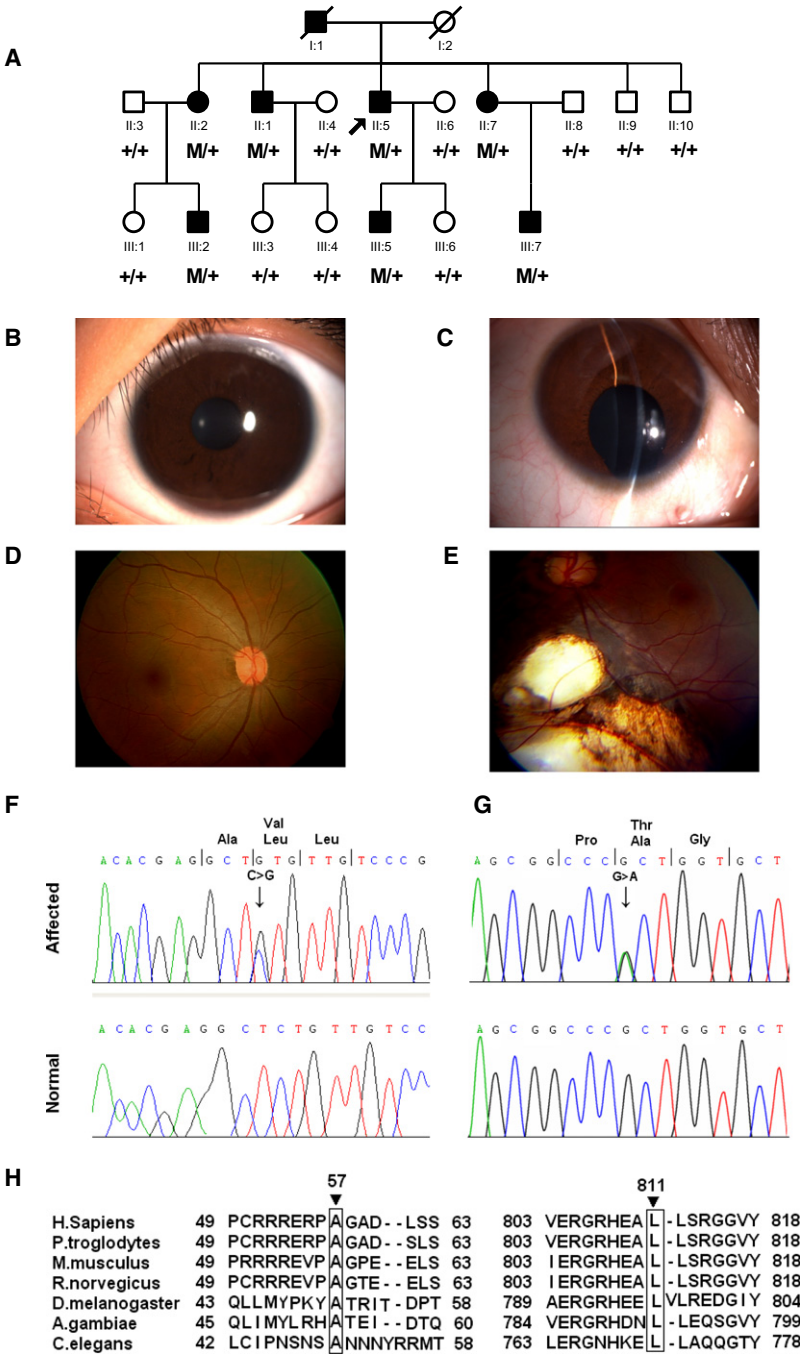

Figure 1. Mutations in *ABCB6* and Clinical Photographs of Patients with Coloboma

(A) Pedigree and segregation of the Leu811Val mutation in the Chinese family affected by coloboma.

(B–E) Photographs of patients with coloboma are compared to those of the unaffected individual. The proband (II:5) of the Chinese family has iris coloboma in the lower papillary margin (C) and inferior chorioretinal coloboma (E). Photographs of the normal iris (B) and fundus (D) of the unaffected individual (II:9) in the same family are also shown.

(F–H) Two mutations in *ABCB6* and their sequencing tracing, including Leu811Val (F) in the Chinese family affected by coloboma and Ala57Thr (G) in the three Indian patients who have microphthalmia and coloboma. A partial sequence of ABCB6 was compared with other species' orthologs (H). Arrows indicate the location of the two mutations identified in patients with ocular coloboma.

Research Foundation and Aravind Eye Hospital. Informed consent was obtained from all participants. All zebrafish studies were conducted under the guidance and approval of the institutional animal care and use committees at Children's Hospital Boston and Sichuan Provincial People's Hospital.

Subjects

This study included one Han Chinese family affected by coloboma. This family has 19 (17 living) family members (Figure 1A). The 600 normal controls are unrelated Han Chinese individuals from the city of Beijing and the Chengdu region of the Sichuan province, China, and they were recruited from the ophthalmology clinics at Peking University Third Hospital and Sichuan Provincial People's Hospital. Additionally, 116 Indian coloboma-affected patients came from the Tamil Nadu region of India and were recruited from the ophthalmology clinic at Aravind Eye Hospital. We chose a separate control population of 200 normal DNA profiles that reflected the race and ethnicity of the 116 coloboma subjects identified in India and screened this population for the identified mutations. Prior to genetic testing, all participants underwent an extensive, standardized examination (performed by ophthalmologists), which included visual-acuity testing, a detailed clinical examination, optical coherence tomography, and ocular imaging. All control individuals underwent an eye exam and showed no signs of coloboma or any other disease in either eye.

Mutation Screening

Peripheral blood was collected from the patients and control subjects. Genomic DNA was isolated with the salt-precipitation method. In the Chinese index family, the disease was linked to chromosomal region 2q35.There are 76 genes and transcripts annotated in the critical interval. Ten of these genes have been screened previously. We performed a mutation screening of the remaining 66 genes and transcripts by using a direct sequencing of PCR-amplified DNA fragments for all exons. We purified these amplified DNA fragments with the QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA, USA), and we used forward and reverse primers to sequence them with the BigDye Terminator v3.1 Cycle Sequencing Kit (ABI Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The *ABCB6* exons of the 116 additional cases from India were also analyzed via the direct sequencing of PCR products.

Sequence Alignments

We used ClustalW (version 1.83) to compare ABCB6 (NP_005680.1) with orthologs of *P. troglodytes* (XP_001161097.1), *M. musculus*

(NP_076221.1), *R. norvegicus* (NP_542149.1), *D. melanogaster* (NP_650503.1), *A. gambiae* (XP_307900.4), and *C. elegans* (NP_001022812.1).

RT-PCR

We used RT-PCR to determine the expression levels of *ABCB6* transcripts in human cell lines, including D407 (retinal pigment epithelial [RPE]) and human embryonic kidney (HEK) 293 cells. We used these same techniques on human tissues to visualize the expression pattern in the heart, retinae, RPE cells, lungs, spleen, kidneys, and blood. These tissues were obtained from a deceased 55-year-old Han Chinese male. Total RNA from the human cell lines and tissues was extracted with Trizol (Invitrogen, Carlsbad, CA, USA) and purified by chloroform extraction and isopropanol precipitation. Reverse transcription was performed with a reverse-transcription kit (Invitrogen, Carlsbad, CA, USA). The forward and reverse primers 5′-GGTGGTGGGCAAGGGCAGAC-3′ and 5′-GGACGAAGTCCAGGGGCCCA-3′, respectively, were used with 100 ng of cDNA for PCR and gave a 432 bp product. The housekeeping gene *β-Actin*, forward primer 5′-AGCGAGCAT CCCCCAAAGTT-3′, and reverse primer 5′-GGGCACGAAGGCTC ATCATT-3′ were used as an internal control to produce a 285 bp product. All RT-PCR products were confirmed by direct sequencing.

For zebrafish RT-PCR, we homogenized ten embryos in Trizol reagent (Invitrogen, Carlsbad, CA, USA) by using a 25 gauge needle. We purified total RNA by chloroform extraction and isopropanol precipitation. We synthesized cDNA from 500 ng of total RNA by using a reverse-transcription kit (Invitrogen, Carlsbad, CA, USA). Forward primer 5′-GGAGTTGTACGGCGTGAGTT-3′ and reverse primer 5′-ACGTCTCGCTCGTCTTCATT-3′ were used with 100 ng of cDNA for PCR. The housekeeping gene *β-Actin*, forward primer 5′-GGACGCCATCCTGAAATA-3′, and reverse primer 5′-CCTGGGAGAACGGTAAAA-3′ were used for PCR as an internal control and gave a 411 bp product. DNA sequencing analysis confirmed all PCR products.

Cell Transfection

The forward 5′-GGGGTACCCCATGGTGACTGTGGGCAACTAC-3′ and reverse 5′-TGCTCTAGAGCACCGTTCCATGGTCTGAGGC-3′ primers contained KpnI and XbaI restriction sites, which were designed for the amplification of WT *ABCB6* cDNA. The amplified cDNA was then digested with KpnI and XbaI and cloned into the KpnI and XbaI sites of the pcDNA3.1(+)/Myc-His A (Invitrogen, Carlsbad, CA, USA) that included a Myc-His tag at the C terminus. The Leu811Val and Ala57Thr mutations were introduced into the WT *ABCB6* pcDNA3.1(+)/Myc-His A construct by PCR-based site-directed mutagenesis. The recombinant plasmids were purified with a QIAGEN plasmid isolation kit (QIAGEN, Valencia, CA, USA) and were confirmed by restriction-enzyme digestion and DNA sequencing.

Cultured D407 cells were propagated in Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. We transfected the cells with recombinant WT or mutant ABCB6 along with either pDsRed2-ER (a marker specific to the endoplasmic reticulum [ER]) or pDsRed2-Mito (a marker specific to mitochondria) (Clontech Laboratories, Mountain View, CA, USA) by using Lipofectamine Reagent 2000 according to the protocol of the manufacturer (Invitrogen; Carlsbad, CA, USA).

Immunostaining and Fluorescence Microscopy of ABCB6 and Cell Compartments

We performed immunostaining by incubating the transferred membrane with Myc monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C, and we then detected the binding Myc antibody by using a second FITC-conjugated anti-mouse antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). For the Golgi apparatus colocalization study, transfected cells were stained with anti-GM130, a Golgi apparatus marker, per the protocol of the manufacturer (Sigma-Aldrich; St. Louis, MO, USA). Images were collected with an Olympus IX70 confocal laser-scanning microscope.

Zebrafish Husbandry

Adult and embryonic strains of albino and AB zebrafish were maintained at 28.5°C on a 14 hr light/10 hr dark cycle, and embryos were produced by natural mating.

Whole-Mount RNA In Situ Hybridization in Zebrafish

We generated the digoxigenin-labeled antisense cRNA probes according to the protocol of the manufacturer (Roche Applied Science, Mannheim, Germany) by using the *abcb6* cDNA templates. The whole-mount in situ hybridization of albino embryos was performed as previously described.[16] Probe hybridization was detected by color development with Pierce NBT/BCIP 1-Step Solution.

Gene Knockdown and Rescue in Zebrafish

abcb6 ATG-inhibition (5′-CACAGAAACTCTTCATCTCCACCAT-3′) (abcb6^{MO1}), *abcb6* splice-blocking (5′-TGCTACCAGCAAGCG TACCTGTTGC-3′) (abcb6^{MO2}), and standard control (5′-CCTCTTA CCTCAGTTACAATTTATA-3′) morpholinos (MOs) were purchased from Gene Tools (Philomath, OR, USA) and were injected into the egg yolk of 1–2-cell-stage Oregon AB embryos. For rescue studies, MOs were coinjected into 1–2-cell-stage zygotes with either wild-type (WT) or mutant *ABCB6* mRNA; these zygotes were expressed in pcDNA3.1(+)/Myc-His A and were purified by a scriptMAX Thermo T7 Transcription Kit (Toyobo, Osaka, Japan).

Immunoblotting

To verify the *ABCB6* mRNA expression in the embryos, we homogenized ten embryos in a lysate buffer containing 1% Triton X-100, 0.01% SDS, 0.05 M Tris-HCl, and 0.001 M EDTA (pH 7.5). The embryo lysates were centrifuged at 4000 rpm for 5 min, and supernatants were used for electrophoresis in a 10% polyacrylamide gel. The proteins were transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA) and were blocked for 1 hr at room temperature with 5% skim milk in Tris-buffered saline containing 0.05% Tween 20 (TBST). The membrane was incubated overnight at 4°C with the Myc monoclonal antibody and was diluted 1:1000 in 5% milk containing TBST. It was then probed with peroxidase-conjugated anti-mouse antibody (1:4000 dilution in TBST, Amersham Biosciences, NJ, USA) for 1 hr and was developed with an ECL detection kit (Amersham Bioscience, NJ, USA) according to the manufacturers' protocol.

Results

Identification of *ABCB6* Mutations

We initially studied a large Chinese family with both well-characterized iris coloboma in the lower papillary

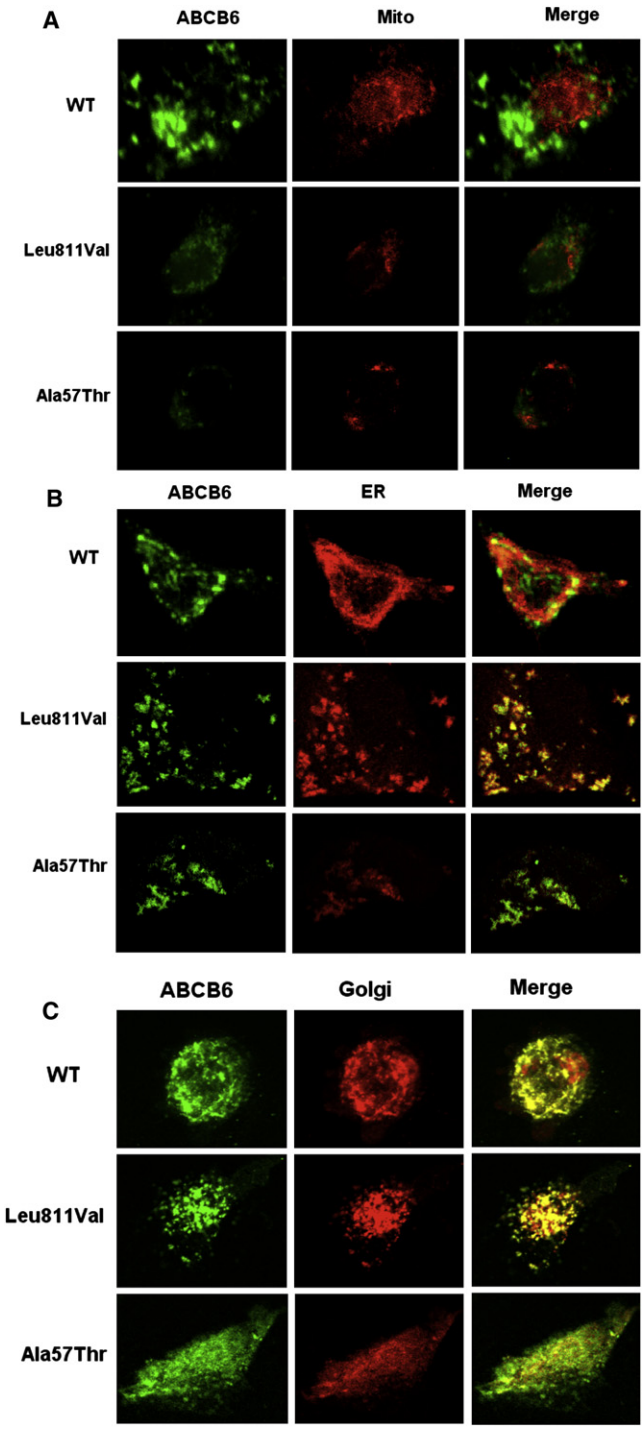

Figure 2. Subcellular Localization of ABCB6 in RPE Cells
Immunofluorescent images of the RPE cell that transiently expresses Myc-tagged ABCB6 and markers for subcellular compartments are shown. Neither WT nor mutant ABCB6, both in green, colocalized with a mitochondrial maker, in red (A). Both WT and mutant ABCB6, in green, colocalized with an ER marker, in red (B), and with a Golgi maker, in red (C).

margin and inferior chorioretinal coloboma[17] (Figures 1A–1E). All of the known genes and/or loci associated with coloboma were examined for mutations. When no mutations in known coloboma-associated genes were identified, we then performed whole-genome linkage analysis and mapped the disease-associated gene in our index family to chromosomal region 2q35, as in our previous report.[17] There are 76 genes and transcripts annotated within the identified interval. We sequenced each exon of these 76 genes from the family's proband (II:5) (Figure 1A) and identified a single nucleotide change in *ABCB6* (MIM 605452) (c.2431C>G). This was the only sequence change that completely cosegregated with the disease phenotype in our index family (Figure 1A). This nucleotide change was absent in DNA samples from 600 controls that were matched for Chinese ethnicity. The nucleotide alteration results in a change of leucine to valine at position 811 (Leu811Val) of *ABCB6* (Figure 1F). Given an autosomal-dominant mode of inheritance with full penetrance and a disease allele frequency of 0.0001, we obtained a LOD score of 3.2 at $\theta = 0$ for this change.

We collaborated with our Indian colleagues, who had a large cohort of DNA samples from subjects with a coloboma phenotype. Initially, we sequenced nine genes (*PAX6*, *SHH*, *GDF3*, *RBP*, *CHX10*, *MAF*, *SOX2*, *OTX2*, and *RAX*) previously shown to produce a coloboma phenotype in 116 sporadic-coloboma samples obtained from patients of Indian decent. These patients were known to have microphthalmia with iris and chorioretinal coloboma (MAC) (63 cases), isolated coloboma (21 cases), and aniridia (32 cases). No mutations were identified in these nine genes in any of these samples.

We then sequenced the *ABCB6* exons in these 116 DNA samples. We identified a substitution in three unrelated patients with coloboma. This substitution was absent in a separate set of 200 DNA samples that were used as ethnically matched controls. This mutation occurred in exon 1 (c. 169 G>A), and the nucleotide change resulted in an alanine to threonine amino acid change at position 57 (Ala57Thr) of ABCB6 (Figure 1G). No additional sequence changes were identified in the coding regions of the 76 genes within the disease interval mapped in this index family. The two missense mutations identified in *ABCB6* are located in a conserved region of the protein (Figure 1H). We conclude that mutations in *ABCB6* cause coloboma and that *ABCB6* is the gene we previously mapped to chromosome 2 in our index family.[16]

ABCB6 Subcellular Localization in RPE Cells

We performed cotransfections of ABCB6 with markers for the ER, mitochondria, and Golgi apparatus to identify where in the D407 cells ABCB6 is localized. In RPE cells, we found that both WT and mutant ABCB6 are located primarily in the ER and Golgi apparatus (Figure 2). This indicated that the alterations in ABCB6 do not affect the protein's subcellular localization for the majority of the cells transfected with mutant ABCB6 constructs.

ABCB6 Expression in the Human Retina and RPE Cells

To investigate whether *ABCB6* is expressed in the eye, we examined *ABCB6* expression in different human cell lines and tissues by using RT-PCR. *ABCB6* expression in retinal

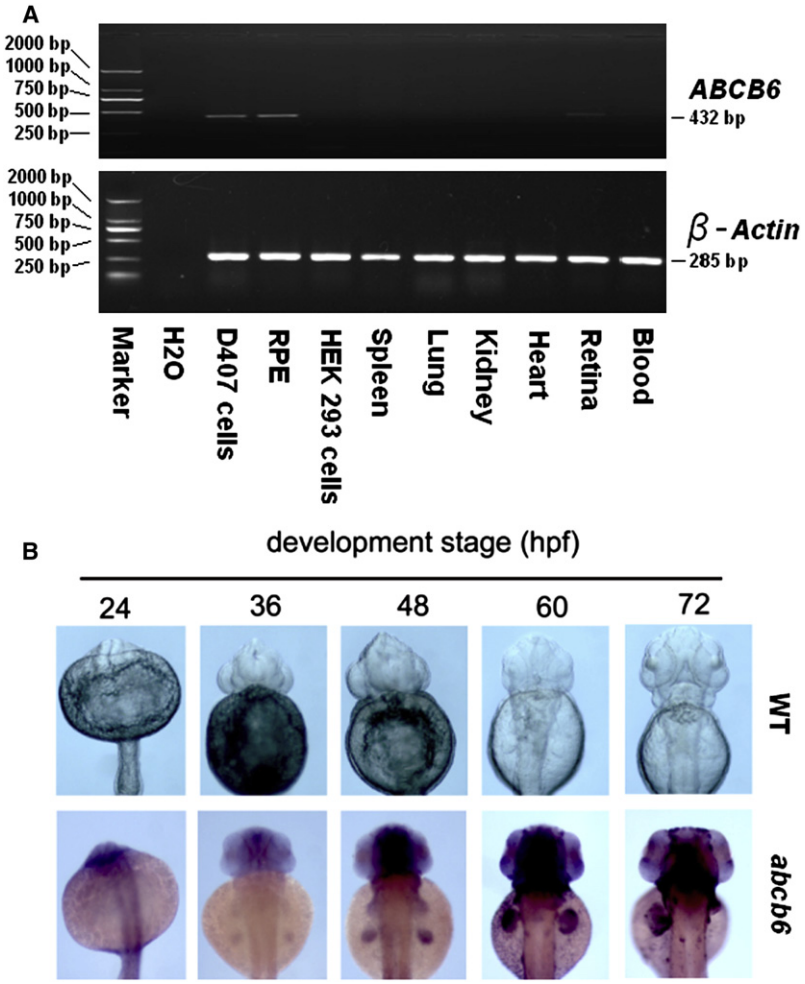

Figure 3. Expression of *ABCB6* in Cell Lines and Tissues and Whole-Mount RNA In Situ Hybridization in Zebrafish

(A) RT-PCR analyses of *ABCB6* expression in D407 and HEK 293 cells and the human spleen, lungs, kidneys, heart, and blood gave a 435 bp product. β-Actin was used as an internal control for cDNA quantification and gave a 285 bp product.

(B) Dorsal views of embryos from 24–72 hpf are shown. WT (unstained) embryos were used as controls. The transcript for *abcb6* was expressed in developing CNS tissue, including the eyes, at 24 hpf and later stages. The expression of abcb6 is detected circumferentially around the lens at 36 and 48 hpf. In addition, *abcb6* is expressed in developing pectoral fins.

and RPE cells is much higher than in other tissues studied (Figure 3A).

Morpholino Knockdown of *abcb6* in Zebrafish and Rescue Studies

To test the hypothesis that disruption of the normal function of ABCB6 can cause coloboma, we developed a zebrafish model to study eye development. We performed whole-mount in situ hybridization to detect the expression of the *abcb6* transcript in early embryogenesis by using albino strains, which lack melanin pigmentation. The *abcb6* transcripts are expressed in the developing CNS tissue, including that of the eyes, at 24 hr postfertilization (hpf) and later (Figure 3B). Expression of *abcb6* is detected circumferentially around the lens at 36 and 48 hpf (Figure 3B). This suggests a function for abcb6 in eye development. In addition, *abcb6* is expressed in developing pectoral fins (Figure 3B). We performed MO knockdown of *abcb6* by using two specific antisense oligonucleotides targeting *abcb6*. The first antisense oligonucleotide blocks translation initiation by targeting the 5′ UTR through the first 25 bases of the coding sequence of *abcb6* (abcb6^{MO1}). The second antisense oligonucleotide modifies pre-mRNA splicing in the nucleus by targeting splice junctions of *abcb6* (abcb6^{MO2}). The standard control MOs were obtained from Gene Tools (Philomath, OR, USA). We used RT-PCR and DNA sequencing to verify the splicing alterations for abcb6^{MO2} (Figure 4A).

We injected 250 zebrafish embryos with either abcb6^{MO1}, abcb6^{MO2}, or control MOs, and the embryos treated with either abcb6^{MO1} or abcb6^{MO2} showed developmental delay compared to those treated with the control MOs (Figure 4E). After 36 hpf, coloboma-related eye defects, which replicated the clinical phenotypes in our patients, were observed in morphants generated by both MOs (Figures 4E and 4F) but were not seen in the control group (Figures 4C and 4D). When zebrafish were stained with 4′,6-diamidino-2-phenylindole, microscope scanning showed that the defect caused by MO knockdown of abcb6 translation was iris coloboma in the lower papillary margin and resulted in a keyhole-like shape of the pupil, as was seen in human patients in our index family (Figure 5). The phenotype of altered eye development induced by either of the *abcb6*-specific morpholinos can be corrected when the embryos are coinjected with WT ABCB6 (Figures 4G and 4H), but not with either of the mutant *ABCB6* mRNAs (Figures 4I–4L). Injection of either abcb6^{MO1} or abcb6^{MO2} caused an abnormal ocular phenotype in 88% or 90% of the fish, respectively (Figure 4M). Coinjection of WT *ABCB6* mRNA with either abcb6^{MO1} or abcb6^{MO2} resulted in a normal phenotype in 85% or 86% of the fish, respectively, whereas coinjection with the same amount of mutant *ABCB6* mRNA caused approximately 25% of the fish to have a normal phenotype (Figures 4G–4M). Immunoblotting with the Myc antibody allowed us to verify that there were equal amounts of WT and mutant ABCB6 translated from the injected mRNA in the embryos (Figure 4B). These results demonstrate that the effect is specifically caused by the knockdown of endogenous abcb6 in zebrafish and suggests that the mutations in *ABCB6* might cause colobomas in humans.

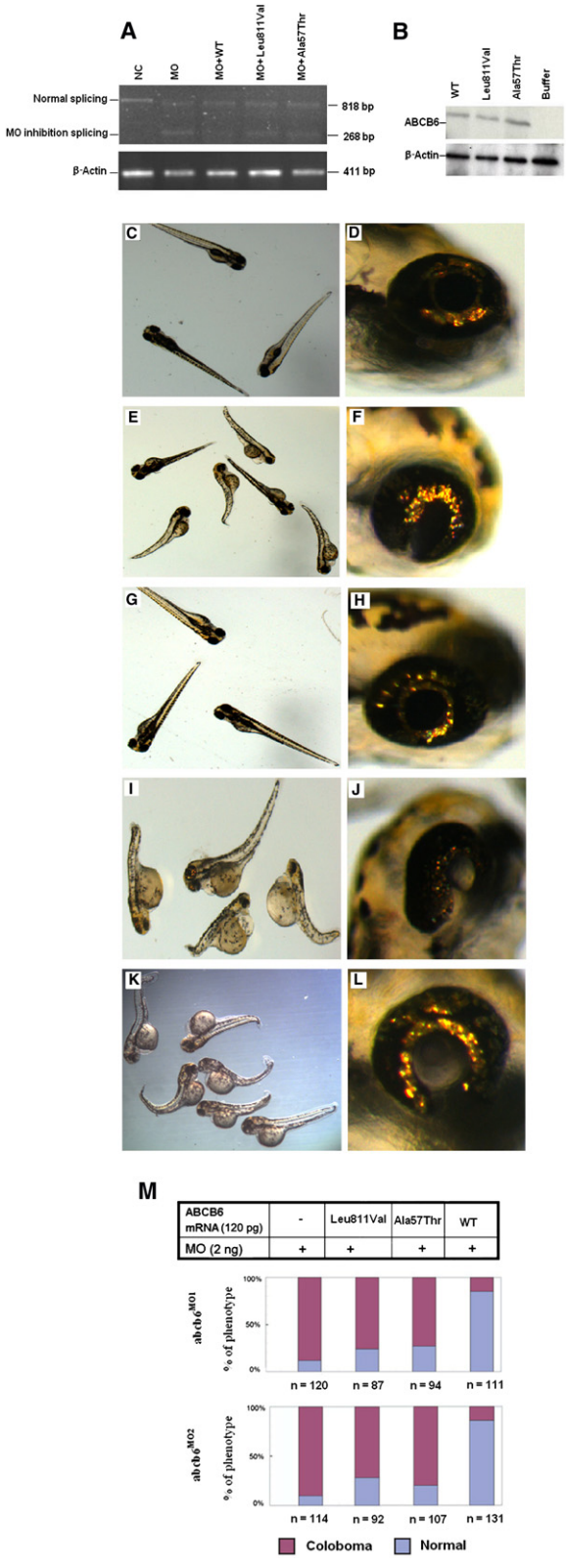

Figure 4. Zebrafish Eye Development Disturbed by Knockdown of *abcb6* Expression and Rescue of *abcb6* Morphants

(A) RT-PCR verified that abcb6^{MO2} altered normal *abcb6* splicing by deleting exon 2. This deletion results in an abnormal 268 bp product as opposed to a normal 818 bp product.

(B) Immunoblotting with the Myc antibody verified that equal amounts of abcb6 produced from the WT and mutant *ABCB6* mRNA were coinjected with MOs in zebrafish. β-Actin was used as an internal control.

(C–L) Compared with zebrafish embryos treated with standard control MOs (C and D), zebrafish embryos treated with abcb6^{MO2} show coloboma and retarded development in 96 hpf (E and F). These phenotypes can be rescued by the coinjection of WT *ABCB6* mRNA (G and H), but the phenotypes could not be rescued by coinjection of the human mRNA containing either the Leu811Val (I and J) or the Ala57Thr (K and L) mutation.

(M) Graph depicting the proportions of embryos with coloboma associated with the injection of either abcb6^{MO1} or abcb6^{MO2} and the proportions rescued by coinjection with WT and mutant *ABCB6* mRNA.

In the zebrafish studies, in which we knocked down expression of *abcb6*, there was no observable phenotype, such as fluorescence or porphyrin accumulation, that would suggest a role for WT or mutant *abcb6* in tetrapyrrole transport. In our studies, there was minimal staining for *abcb6* mRNA expression in the developing liver and blood island (intermediate cell mass), suggesting that if there is a role for ABCB6 in erythroid development, it is minimal. This would be contrary to what has been shown previously for components of the heme biosynthetic pathway.

Discussion

The formation and closure of the optic fissure requires precisely coordinated sculpting and folding of the epithelial tissue so that the edges of the optic fissure can align, converge, and fuse. This early developmental process of the eye is controlled by a complex network of transcriptional factors, cell-cycle regulators, and diffusible signaling molecules.[18] Ocular coloboma occurs if optic closure is disrupted as a result of poor regulation of this complex network. Transcription factors such as PAX6, SOX2, OTX2, RAX, and SIX3 interact and provide transcriptional oversight to other coloboma-associated mutations in genes that play a pivotal role in spatial and temporal development of the eye.[8,10–12,15,19] Here, we identified two mutations (Leu811Val and Ala57Thr) in ABCB6. Comparative analyses of *ABCB6* with the same gene in other species showed that Leu811 and Ala57 are highly conserved, implying that these two residues are key to normal biological function. The phenotype caused by disruption of *ABCB6* might be explained by haploinsufficiency for several reasons. (1) The two identified mutations in *ABCB6* are heterozygous in our patients with coloboma (Figure 1B). (2) *ABCB6* is expressed in the human eye, and *abcb6* is expressed in the zebrafish CNS, including the eyes. Decreased mRNA expression caused by morpholino knockdown in zebrafish replicates the coloboma phenotype in the index cases. However, approximately 50% of normal mRNA can be detected in the morphants at 48 hpf (Figure 4A). (3) Finally, the knockdown phenotype can be corrected with the coinjection of WT, but not mutant, *ABCB6* mRNA.

ABCB6 belongs to the ABC transporter family, which is involved in the active transport of phospholipids, ions,

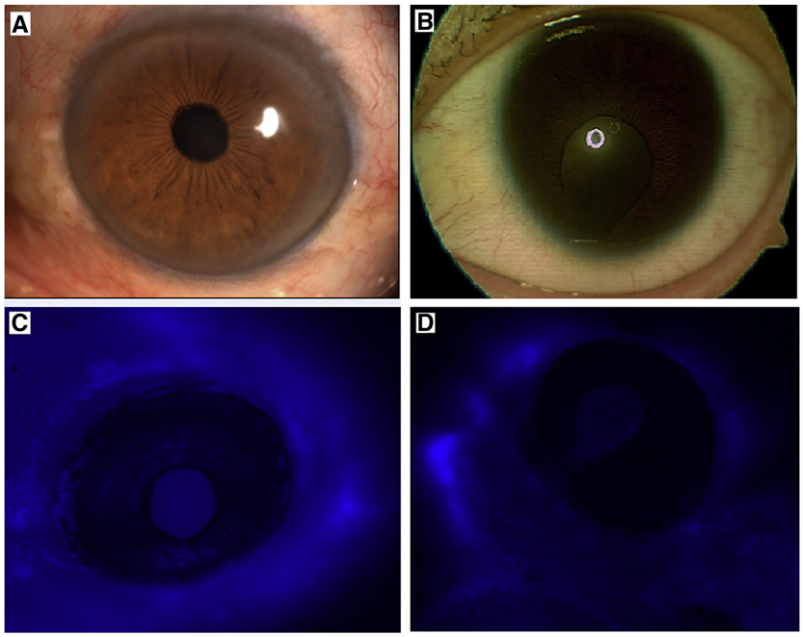

Figure 5. Iris Coloboma Produced by Morpholino Knockdown of *abcb6* in Zebrafish Compared with the Patients with Coloboma in the Chinese Family

(A) A normal iris is shown in an unaffected individual in the same family as the control. (B) A pupil shaped like a keyhole is seen in an affected patient in the Chinese family afflicted with coloboma.

(C) The eye of a fish that was treated with a control morpholino.

(D) Morphants treated with abcb6^{MO1} result in a keyhole-shaped pupil similar to that seen in the patients with coloboma.

peptides, steroids, polysaccharides, amino acids, and bile acids and can be used to transport nonphysiologic compounds such as pharmaceutical drugs and other xenobiotic compounds.[20–23] Previous studies have indicated that mutations in 14 ABC transporters are associated with 13 different Mendelian disorders, and genetic variants in ABC transporters are associated with complex disorders in humans.[20,24] Among these disorders, macular dystrophy can be caused by mutations in ABCA4 (MIM 601691).[25]

The cellular localization of ABCB6 might be cell-type dependent. Previous studies have indicated that ABCB6 localizes to the outer mitochondrial membrane[26,27] and is a mammalian mitochondrial porphyrin transporter.[26] However, recent studies revealed that ABCB6 is glycosylated in multiple cell types and is also found in the classical secretory pathway, which includes the ER, Golgi apparatus, and plasma membrane.[27,28] Consistent with these findings, we found that ABCB6 localizes to the classical secretory pathway, including the ER and Golgi complex, indicating additional ABCB6 functions beyond its proposed role as a porphyrin transporter in the mitochondria of RPE cells.

In humans, ABCB7 (MIM 300135) and ABCB6 (MTABC3) have been identified as close homologs of ATM1, implying a function of ABCB6 in the synthesis of Fe-S clusters and/or iron homeostasis. Metal ions have been shown to affect the development of the eye: Ca^{2+}, Zn2+, Mg2+, and Fe2+ are essential trace elements required for normal eye development. However, heavy metals such as Ag, Au, Pb, and Hg are implicated in structural and physiological damage in the mammalian eye. Therefore, it is possible that disruption of the normal biological functions of ABCB6 results in abnormal metal homeostasis, which is detrimental to proper eye development. During fetal development, retinoic acid has a function in limb development and formation of the heart, eyes, and ears.[29] Our hypothesis is that ABCB6 transports a signaling molecule involved in eye development. Any alteration in specificity or rate might lead to coloboma.

Like PAX6 and SHH, ABCB6 is expressed elsewhere in the developing CNS, but mutations in *ABCB6* are mainly associated with eye anomalies, presumably because ABCB6's function can be compensated for elsewhere in the CNS.[1] Alternatively, the differential tissue requirements for ABCB6 represent the demand for the metabolite that is transported, and a 50% reduction is not enough to cause abnormal development in the other tissues. Given that abcb6 is expressed in the fin buds in zebrafish, it is possible that mutations in this gene cause, in addition to coloboma, limb and/or skeletal anomalies comparable to the observed phenotypes resulting from coloboma-associated genetic mutations in *GDF3* and *GDF6* (MIM 601147).[6,7]

Defining the physiologic role of ABC transporter function will require further studies, including disease-specific mutation screening, which links organs to specific phenotypes, and mechanistic dissection of transporter function. A directed screen to determine the role of ABCB6 in eye development and to determine the mechanism leading to ocular coloboma will provide important insight into developmental signaling that leads to optic malformations.

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Web Resources

The URLs for data presented herein are as follows:

GenBank, www.ncbi.nlm.nih.gov/Genbank/
Online Medelian Inheritance in Man (OMIM), www.omim.org
UCSC Genome Browser, www.genome.ucsc.edu/cgi-bin/hgGateway

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